37.1

◉ Sheet ○ Slides

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This sheet will cover the first topic discussed in lecture 37: **pyrimidines**

I. Pyrimidines synthesis:

Pyrimidines are nitrogenous bases that are composed of a single hexagon ring.

- The main difference in their synthesis from purines is that the base is **built before it's added to the sugar** (NOT built ON the sugar).

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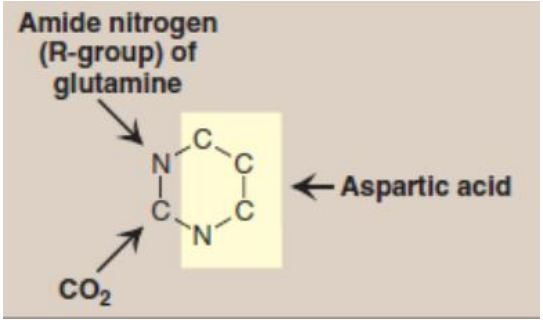
a. De novo synthesis:



- **Sources of atoms:** 2 amino acids (glutamine, aspartate) and CO_2. *In addition to PRPP later*

- **Pathway:**

  Step 1: gathering atoms

  Amino group of glutamine + a CO_2 molecule + 2 ATP molecules are used to form a **carbamoyl phosphate** by **carbamoyl phosphate synthetase II**

  this is the regulated step of pyrimidine synthesis

  CPS II is inhibited by UTP (the end product of this pathway) and is activated by PRPP

  Step 2: adding aspartate

  Aspartate enters and attaches to carbamoyl phosphate displacing the phosphate and producing **carbamoyl aspartate.**

  *now we have the *six* atoms of the ring ready, *one* from glutamine, *one* from CO2 and, *four* from aspartate*

Step 3: ring formation

By the enzyme **dihydroorotase**, the terminal atoms are bonded together to close the ring forming dihydroorotate and an H2O molecule is released.

* The first three enzymic activities in this pathway (**CPS II, aspartate transcarbamoylase, and dihydroorotase)** are three different catalytic domains of a single polypeptide chain (CAD)*

Step 4: oxidation on dihydroorotate

Dihydroorotate gets oxidized by **dihydroorotate dehydrogenase** to **orotate** with the reduction of FAD to FADH2.

* This enzyme is associated with the inner mitochondrial membrane, all the other enzymes of this pathway are **cytosolic***

- **See? As mentioned earlier, unlike purine nucleotides, pyrimidine rings are completely formed before they're converted to nucleotides by the addition of sugar monomers.**

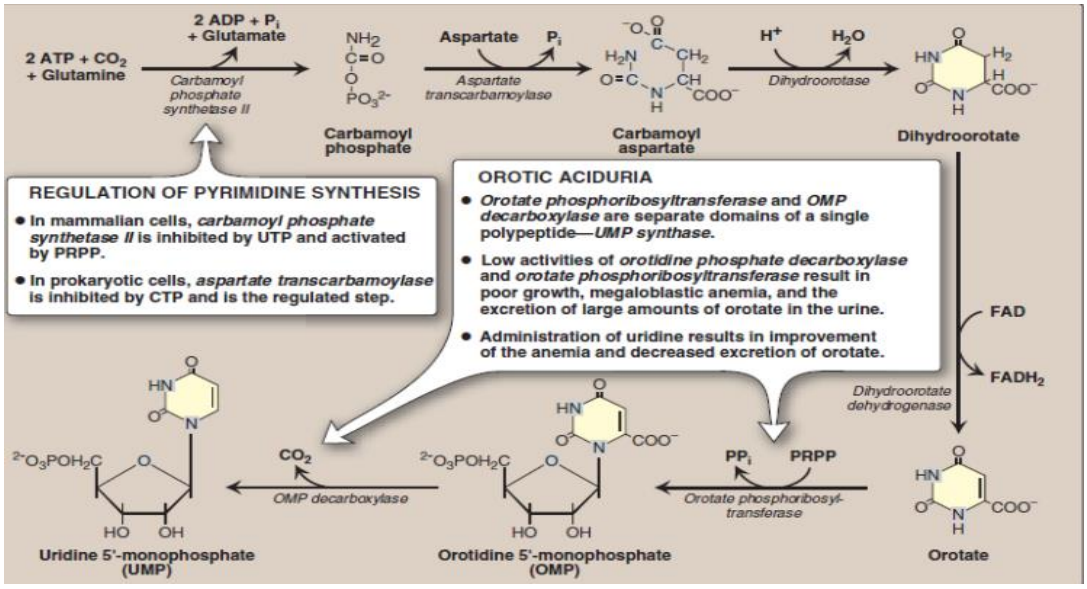
Step 5: sugar addition

PRPP enters and **donates a phosoribose** with the irreversible release of pyrophosphate by Orotate phosphoribosyl transferase , producing **orotidine monophosphate (OMP)**, the **parent pyrimidine mononucleotide.**

Step 6: Pyrimidine nucleotide synthesis (UMP, TMP, CMP)

- **OMP decarboxylase** removes a carboxyl group from the nitrogenous base (it was part of the aspartate when we added it) producing **Uridine monophosphate** (UMP).
- **Orotate phosphoribosyl transferase and orotidylate (OMP) decarboxylase** are catalytic domains of a single polypeptide chain called UMP synthase.

Orotic aciduria, a rare genetic defect, is caused by a deficiency of one or both activities of the bifunctional UMP synthase resulting in **orotic acid in the urine.**



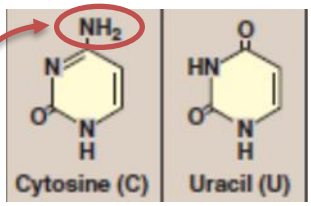
- UMP is a mono nucleotide that can be phosphorylated to di and tri nucleotides which are **used in the synthesis of RNA**. However, UMP is also modified someway to **synthesize TMP and CMP which are used in the synthesis of DNA.**

- **Cytosine and thymine formation:**
- For the synthesis of CTP and TTP, UMP needs to go under some changes.
- UMP is phosphorylated to UDP and then UTP.

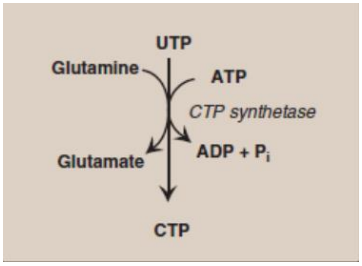
  UTP used in CTP synthesis
- UDP is a substrate for **ribonucleotide reductase**, which generates **dUDP.**
- **dUDP** can be phosphorylated to **dUTP**, which is rapidly hydrolyzed to **dUMP** by **UTP di-phosphatase (dUTPase)**. *dUMP used in dTMP synthesis*

*dUTPase reduces (decrease) the available dUTP for DNA synthesis, thus preventing incorporation of uracil into DNA.

➢ **Cytosine: (UTP → CTP)**

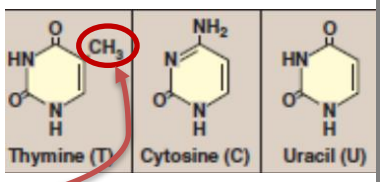


The difference between cytosine and uracil is this **amino group**; so, **glutamine** donates this amino group becoming glutamate with the help of **CTP syntheTASE** (ATP requiring enzyme).



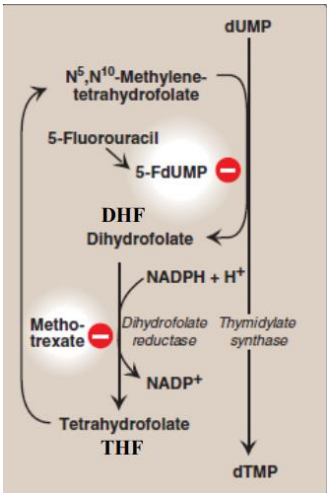
Some CTP is dephosphorylated to CDP (which is a substrate for **ribonucleotide reductase** - produces dCDP which can be phosphorylated to dCTP for DNA synthesis).

➢ **Thymine: (dUMP → dTMP)**



We start with **deoxy**-UMP to produce **deoxy**-TMP by the action of an enzyme called **thymidylate synthASE**.

The difference between uracil and thymine is this **methyl group.**



Thymidylate synthase **methylates** uracil to form thymine using **N^5, N_{10}-methylene-tetrahydropholate** as the source of the methyl group.

This results in the conversion of **N^5, N_{10}-methylene-tetrahydropholate** to **dihydrofolate.**

Therefore, we need to recycle it back to **tetrahydrofolate** in order to reuse it, and that's accomplished by **dihydrofolate reductase**, which adds two hydrogens reducing it back to **tetrahydrofolate** with oxidation of NADPH to $NADP^+$.

➢ Some anti cancerous agents target this process to inhibit the growth of tumor cells:

- **Thymidylate synthase inhibitors** include **thymine analogs** such as **5-Fluorouracil** (antitumor agents).

  5-Fluorouracil (suicide inhibitor) is converted to 5-Fd**UMP** that **permanently** binds and **inactivates thymidylate synthase.**

- **Methotrexate** inhibits dihydrofolate reductase.

  Methotrexate (folate analog) reduces the amount of THF, thus inhibits **purine** synthesis and **prevents methylation of dUMP to dTMP**, resulting in **DNA synthesis inhibition and cell growth slow down**.

How does the cell distinguish whether this carbamoyl phosphate is going into nucleotide synthesis or urea cycle? (CPS= carbamoyl phosphate synthetase)

- Mainly, depending on the **site** of the reaction.

(CPS I) is active in **hepatocytes** - specifically in the **mitochondria-**. The **pathway** it's involved in is **the urea cycle** and the added **amino group's source is ammonia**.

(CPS II) is active in the **cytosol** of **many different cells**, the source of the amino group it adds is **glutamine** and the pathway it's involved in is **pyrimidine synthesis**.

	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ-Amide group of glutamine
Regulators	Activator: N–acetyl-glutamate	Activator: PRPP Inhibitor: UTP

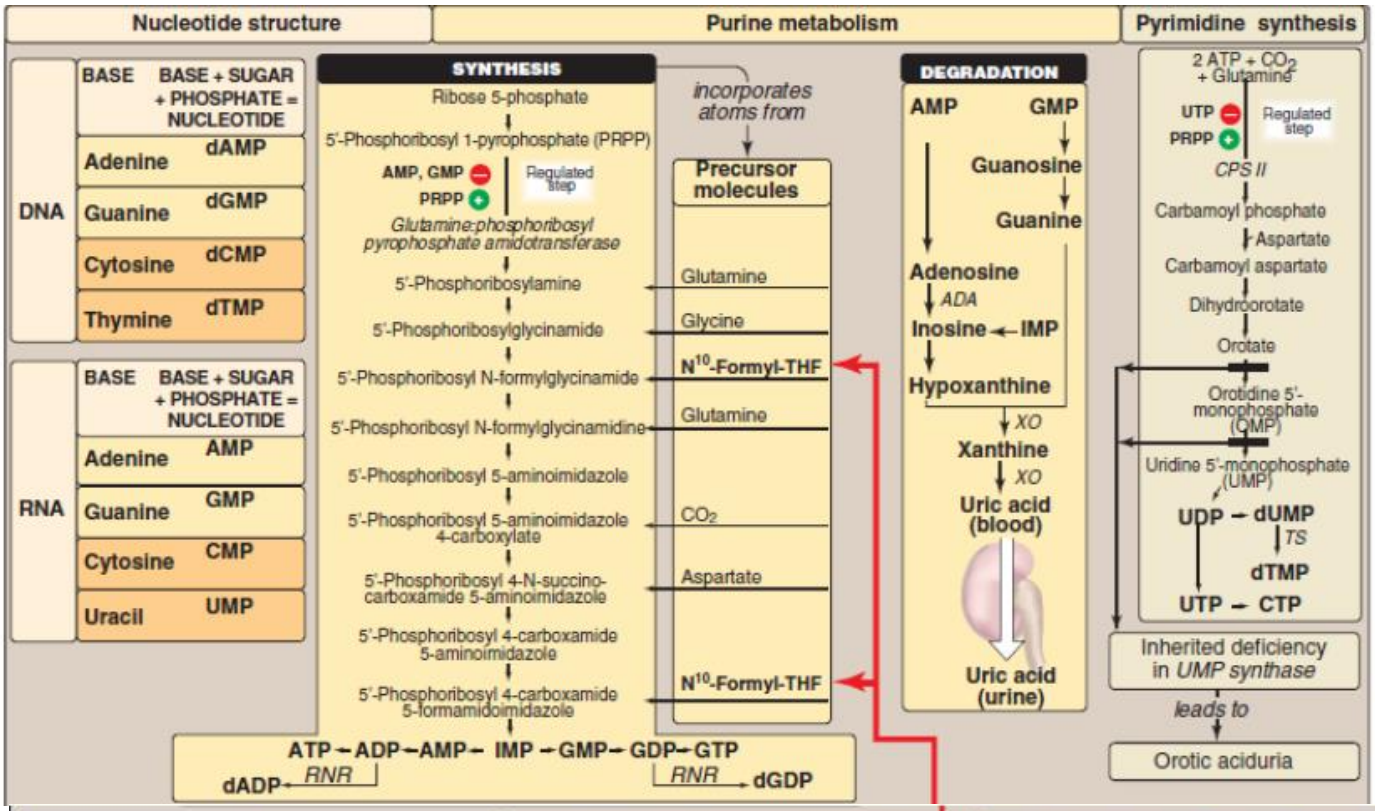
b. salvage synthesis:

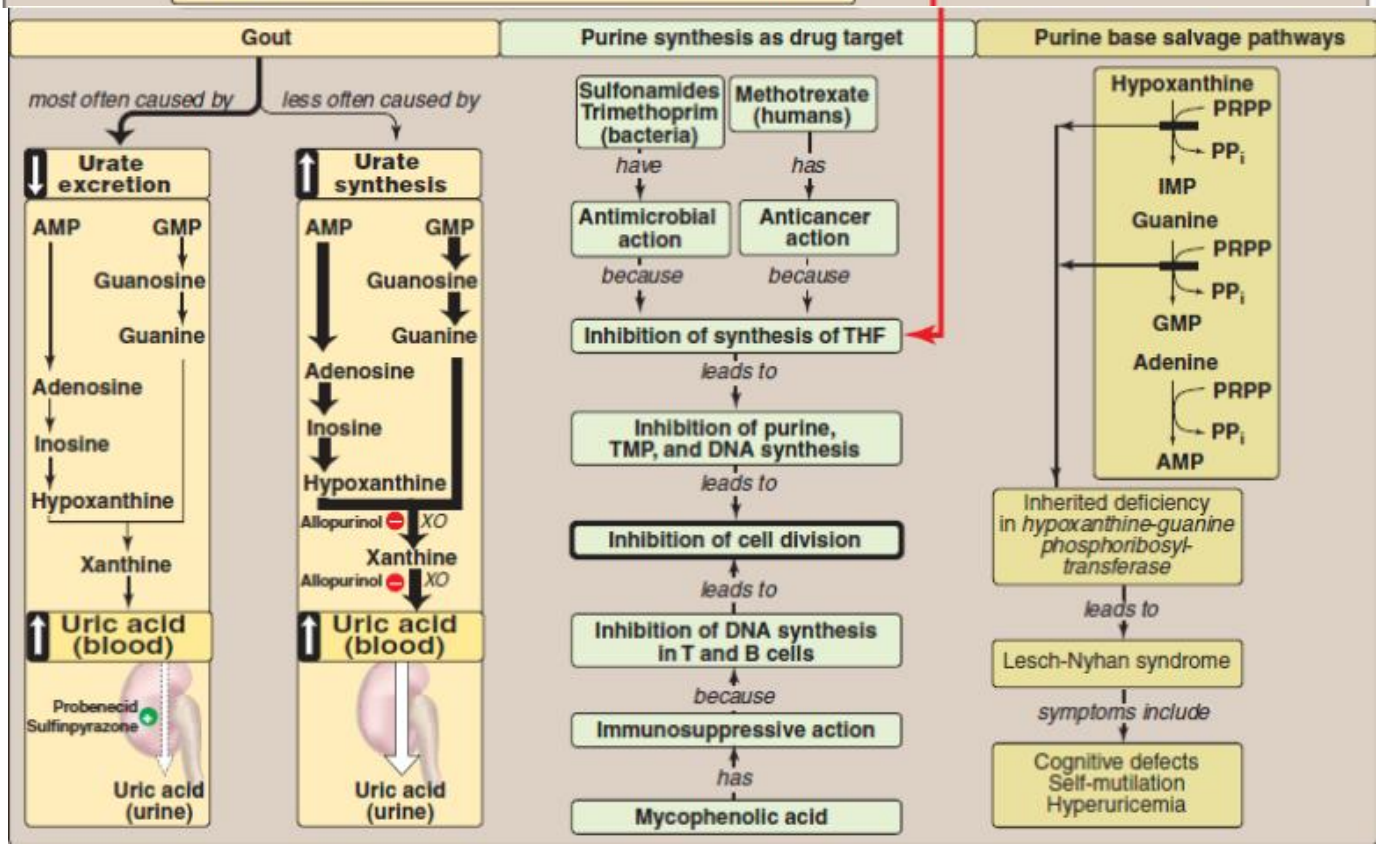
phosphorylation of nucleosides of pyrimidines (Nitrogenous base + sugar) by a kinase enzyme with the use of ATP gives us **salvage synthesized nucleotides**

II. Degradation

Simply, the pyrimidine ring is opened and degraded to highly soluble products: **β-alanine and β-aminoisobutyrate** with the production of NH_3 and CO_2.

Good luck :)


Nucleotide structure
Purine metabolism
Pyrimidine synthesis
DNA
BASE
BASE + SUGAR + PHOSPHATE = NUCLEOTIDE
Adenine
dAMP
Guanine
dGMP
Cytosine
dCMP
Thymine
dTMP
RNA
BASE
BASE + SUGAR + PHOSPHATE = NUCLEOTIDE
Adenine
AMP
Guanine
GMP
Cytosine
CMP
Uracil
UMP
SYNTHESIS
Ribose 5-phosphate
5'-Phosphoribosyl 1-pyrophosphate (PRPP)
AMP, GMP
PRPP
Regulated step
Glutamine:phosphoribosyl pyrophosphate amidotransferase
5'-Phosphoribosylamine
5'-Phosphoribosylglycinamide
5'-Phosphoribosyl N-formylglycinamide
5'-Phosphoribosyl N-formylglycinamidine
5'-Phosphoribosyl 5-aminoimidazole
5'-Phosphoribosyl 5-aminoimidazole 4-carboxylate
5'-Phosphoribosyl 4-N-succino-carboxamide 5-aminoimidazole
5'-Phosphoribosyl 4-carboxamide 5-aminoimidazole
5'-Phosphoribosyl 4-carboxamide 5-formamidoimidazole
incorporates atoms from
Precursor molecules
Glutamine
Glycine
N10-Formyl-THF
Glutamine
CO2
Aspartate
N10-Formyl-THF
ATP – ADP – AMP – IMP – GMP – GDP – GTP
dADP
RNR
RNR
dGDP
DEGRADATION
AMP
GMP
Guanosine
Guanine
Adenosine
ADA
Inosine
IMP
Hypoxanthine
XO
Xanthine
XO
Uric acid (blood)
Uric acid (urine)
2 ATP + CO2 + Glutamine
UTP
PRPP
Regulated step
CPS II
Carbamoyl phosphate
Aspartate
Carbamoyl aspartate
Dihydroorotate
Orotate
Orotidine 5'-monophosphate (OMP)
Uridine 5'-monophosphate (UMP)
UDP – dUMP
TS
dTMP
UTP – CTP
Inherited deficiency in UMP synthase
leads to
Orotic aciduria


Gout
most often caused by
less often caused by
Urate excretion
AMP
GMP
Guanosine
Guanine
Adenosine
Inosine
Hypoxanthine
Xanthine
Uric acid (blood)
Probenecid
Sulfinpyrazone
Uric acid (urine)
Urate synthesis
AMP
GMP
Guanosine
Guanine
Adenosine
Inosine
Hypoxanthine
Allopurinol
XO
Xanthine
Allopurinol
XO
Uric acid (blood)
Uric acid (urine)
Purine synthesis as drug target
Sulfonamides Trimethoprim (bacteria)
Methotrexate (humans)
have
has
Antimicrobial action
Anticancer action
because
because
Inhibition of synthesis of THF
leads to
Inhibition of purine, TMP, and DNA synthesis
leads to
Inhibition of cell division
leads to
Inhibition of DNA synthesis in T and B cells
because
Immunosuppressive action
has
Mycophenolic acid
Purine base salvage pathways
Hypoxanthine
PRPP
PPi
IMP
Guanine
PRPP
PPi
GMP
Adenine
PRPP
PPi
AMP
Inherited deficiency in hypoxanthine-guanine phosphoribosyl-transferase
leads to
Lesch-Nyhan syndrome
symptoms include
Cognitive defects
Self-mutilation
Hyperuricemia